UNIVERSITY OF PENNSYLVANIA

STUDIES ON THE UTERUS

V. THE INFLUENCE OF THE OVARY ON THE MOTILITY OF THE NON-GRAVID UTERUS OF THE UNANESTHETIZED RABBIT

SAMUEL ROBERT MEANS REYNOLDS

A THESIS

IN PHYSIOLOGY

PRESENTED TO THE FACULTY OF THE GRADUATE SCHOOL IN

PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR

THE DEGREE OF DOCTOR OF PHILOSOPHY

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V. The Influence of the Ovary on the Motility of the Non-Gravid Uterus of the Unanesthetized Rabbit[1,2]

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In a previous study we have called attention to the fact that prior to coitus, unanesthetized female rabbits usually exhibit a marked or a moderate motility of the uterine fistula (Reynolds and Friedman, 1930a). Earlier we called attention to the activity of uterine fistulae of four normal rabbits, over extended periods of time (Reynolds, 1930a). Two of these rabbits had, at one time or another, uteri which were completely quiescent, whereas at other periods there was marked activity which was in striking contrast to the inactive state. In view of these results we were tempted to suggest that a marked type of uterine activity might possibly be associated with heat, and the quiescent condition, with the absence of heat. Although there are now in the literature data obtained on the excised uterus which would seem to support such a view, nevertheless we do not consider that such data were strictly comparable to the results one may obtain in the intact animal. For example, we have shown (Reynolds, 1930b) that the quiescent uterine fistula does not respond to the intravenous injection of pituitary extracts, whereas the excised uterine strip never fails to respond to such preparations. Because of such a discrepancy between the results obtained in the *in vitro* and in the *in vivo* experiments, it seemed advisable to investigate in the intact, non-pregnant animal, the rôle of the ovary in regulating uterine motility. This we have done by determining:

1. The uterine motility associated with oestrus and anoestrus.
2. The uterine motility in the castrate female, and so in the absence of all influence of the internal secretions of the ovary.
3. The response of the uterine fistula of the castrate to the injection of lipoid-soluble and water-soluble oestrogenic extracts.

[1] The expenses of this research have been aided by a grant to Prof. H. C. Bazett from the Committee for Problems on Research of Sex of the National Research Council. The funds were administered by Dr. M. H. Friedman.

[2] The work of this paper has been accepted in partial fulfillment of the requirements for the degree of Doctor of Philosophy in this University.

[3] George Lieb Harrison Fellow in Medical Sciences, 1930–31.

The operative technic and recording procedure which we have developed for recording uterine motility in the unanesthetized chronic rabbit have been described elsewhere (Reynolds, 1930a; Reynolds and Friedman, 1930a), and will not be considered here except as it appears necessary in describing the modifications that were made in the examination of the several points under investigation.

EXPERIMENTAL RESULTS. 1. *Uterine activity associated with oestrus and anoestrus.* The rabbits used for this phase of the work were taken out of our general stock, the care of which we have already described (Reynolds and Friedman, 1930a). It was known, by segregation of the does or by laparotomy, that the ovaries of these animals contained no corpora lutea at the beginning of each experiment, and through the course of each experiment the rabbits were isolated from each other and from the bucks, except at the times when matings were attempted. Fistulae were made, and the animals allowed to recover for four days in one instance, to several weeks in others before an attempt to mate them was made. In every instance a control record of uterine activity was obtained, after which the doe was placed with the buck. Whether or not coitus occurred, was determined by observation and by examination of the vagina for sperm. The ovaries of those does which accepted the male were examined at the end of a period of pseudopregnancy for corpora lutea, as evidence of ovulation.

A summary of the observations which we have made on the correlation of uterine motility with heat or the absence of heat are briefly tabulated in table 1. Here it may be seen that twenty-six does accepted the male, or were known to be in heat (immediately post partum; Hammond and Marshall, 1925). Sixteen of these showed rhythmic, marked activity; eight, moderate activity, and two, feeble activity. Of does not in heat, fourteen refused the male 23 times while showing feeble activity; seven refused the male 12 times while showing moderate activity. No does that showed marked activity ever refused the male.

Of the seventeen does which at one time or another refused the male, three did accept the male later, and so had come on heat. One of these showed no change in the moderate activity of her uterus from July 19th until August 23rd, at which time she had coitus. Another, on the other hand, showed within the space of one week, activity increasing from a feeble irregular sort, to a slow rhythmical, marked type of activity when she accepted the male. Still a third one had coitus when she was exhibiting a feeble, irregular sort of activity. These three animals present several distinct and contrasting histories of uterine activity before coitus, and are not, in the main, in accord with the larger part of our results cited above. A consideration of them, and of the last one in particular, will be deferred until later.

As regards a correlation of the frequency of the occurrence of the uterine contractions, and the presence or absence of heat, there appears to be no regular finding. The intervals between the commencement of the contractions of moderate size in the anoestrus animals have ranged from 20 to 90 seconds; in oestrus animals, from 20 to 60 seconds. Of those animals in oestrus which showed marked activity, the range of the frequencies is more or less 60 to 90 seconds, although one of the does showing the most marked activity was one whose uterus contracted once each 30 seconds. Possibly the most definite correlation apparent from our results is that feeble contractions, as recorded by our method, are quite irregular, and are most

TABLE 1

Summary of data correlating uterine motility with oestrus and anoestrus

Types of activity may be designated in the following manner:

0 = Quiescent condition of uterus in which no activity can be recorded with our system.

\+ = Feeble activity is that which with our system of recording gives records of contractions of ½ inch or slightly less.

++ = Moderate activity is that which varies between ½ inch to 2 inches, but which appears to average 1 inch to 1½ inches.

+++ = Marked activity is that which varies between 2 inches to 4 inches.

++++ = Marked activity, over 4 inches.

In all these classes of activity one may find rhythmical (regular) activity, or arhythmical (irregular) activity. Frequently with feeble activity one sees a sort of undulating movement, constant in rate on any one animal for a short period of time, but varying in rapidity from animal to animal.

TYPE OF ACTIVITY	ANOESTRUS		OESTRUS	
	Number of does	Number of instances	Number of does	Number of instances
0 or +	14	23	2	2
++	7	13	8	8
+++ or ++++	0	0	16	16
Total.....	21	36	26	26

characteristic of anoestrus, while in contrast to this, the activity of does in heat is almost always rhythmical.

2. *Uterine motility in the castrate rabbit.* A brief summary of the data which we have obtained on spontaneous uterine motility following complete ovariectomy is presented in table 2. These data are taken from experiments done in connection with the latter part of this paper. With the exception of the rabbits used in the experiments with the lipoid-soluble Estrogen, we have used only does which have dropped a litter one to fourteen days before castration. By so doing, all the uteri were in the oestral stage at the time of castration. Two series of experiments were performed,

one on "recent" castrates, and the other on "old" castrates. In the former series (26 animals) records were obtained three to six days after castration, in the latter (11 animals) no records were made until three weeks after ovariectomy. In eight rabbits, included in the first series, records of uterine activity were obtained while the does were in heat before castration as well as for three or more days after ovariectomy.

The motility of the uterus, both early and late after castration, may be seen in table 2. The characteristic changes which regularly follow removal of both ovaries may be listed as follows: 1, all rhythmicity is soon lost; 2, a majority of the rabbits exhibit a quiescent uterus or one which shows only feeble irregular activity; 3, these effects on rhythmicity and amplitude of the contractions are pronounced, if not complete, within two to four days following castration; 4, at the expiration of three weeks the uterus is completely quiescent (ten of eleven cases).

TABLE 2

Summary of data correlating uterine motility with absence of ovarian influence: ovariectomy

TYPE OF ACTIVITY (IRREGULAR)	RECENT CASTRATES		OLD CASTRATES	
	Number of does	Number of days castrated	Number of does	Number of days castrated
0	14	3–10	10	20–21
+	7	2–6	1	21
++	5	2–5	0	
+++	0		0	
Total.....	26		11	

The characteristics just described are derived chiefly from isolated observations on rabbits after castration has been performed, with no control runs obtained while the ovaries were yet intact. But the experiments on the eight rabbits in which records were obtained both before and after castration demonstrate that the loss of all but feeble irregular activity must be ascribed to the absence of the ovaries. In these experiments we recorded the motility of the uterus immediately *post partum*, while one or both ovaries were still intact, and again on subsequent days after partial or complete ovariectomy. One to four days *post partum* one ovary was removed and fistulae made, in four rabbits. Uterine activity was recorded on subsequent days, until moderate rhythmic contractions were seen, or until there was no increase in the amplitude of the contractions. Recovery from the effects of the operation seemed to be achieved in the course of two to three days (see fig. 1). The second ovary was removed on the fourth day after the first operation. The pro-

found effect that set in as a result of this second operation, or complete castration, in every instance affecting both the amplitude and rhythm of the contractions, is obvious from the records we have obtained. The decrease in activity persisted until the sixth day of castration at which time these four rabbits were injected with Theelin as described below. Four rabbits were used as controls, in that, at the time the fistula was prepared, both ovaries were left intact. Activity was recorded on the

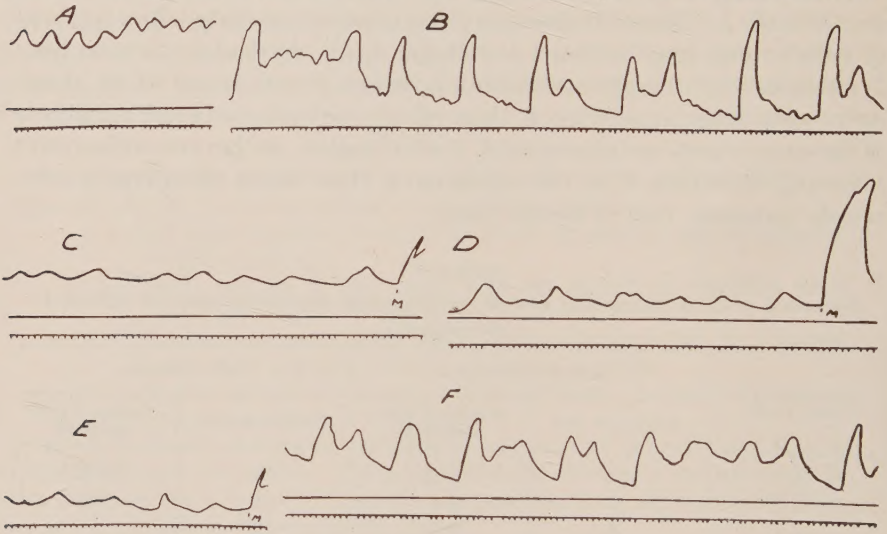

Fig. 1. Rabbit 41. Records showing the motility of the uterus two days after preparation of the fistula, *A*; a return to the oestral type of activity on the third day, *B*; the effect of ovariectomy 2, 4, and 6 days after castration, *C*, *D*, *E* respectively; and 24 hours after replacement therapy with 2 r. u. oestrin per kilogram body-weight, *F*. Protocol of this experiment:

	2/12/31	Litter born; removed immediately.
	2/13/31	Preparation of fistula. Left ovary removed at this time.
A	2/15/31	Activity of uterus two days after operation.
B	2/16/31	Activity of uterus three days after operation. Complete castration by removal of right ovary through flank incision.
C	2/18/31	Activity two days after complete castration.
D	2/20/31	Activity four days after castration.
E	2/22/31	Activity six days after castration. Weight of rabbit, 2.5 kgm. Four intravenous injections of 1.25 r. u. (0.025 cc.) Theelin each at 2½ hour intervals, (total 5 r. u., or 2 r. u./kilo).
F	2/23/31	Record showing return to activity 24 hours after first injection of Theelin. Rabbit killed at once.

Autopsy findings of uterus: good condition; free from all inflammation, infection, perforations and adhesions. Some "uterine milk." Sites of ovariectomies, free from infection; with only slight adhesions.

M, mechanical responses elicited by pressure on abdomen to indicate that the balloon is freely compressible.

One-fifth original size.

second and third days subsequently, and on this latter day, one ovary was removed. No pronounced or persistent effect of the operation was seen. The second ovary of each of these animals was later removed, and the typical castration syndrome developed promptly, to a profound extent. Two does were not castrated but were maintained as controls. They showed persistence of moderate activity in one instance, marked activity in the other, and both showed a willingness to copulate when placed with the male. These results conclusively show that the changes which we have described that are a consequence of castration of a doe in heat are due primarily, if not entirely, to the loss of the immediate influence of the ovaries.

Attention is called to the fact that of all the recent castrates, only one showed a moderate, regular activity; it is the *only* one of our recent castrates that has ever spontaneously accepted the male. This was on the second day following castration during oestrus. Four and six days after castration, the activity of her uterus was both feeble and irregular,—a type of activity which is in agreement with the data obtained from the other seven rabbits of this group of experiments. In all eight, then, we have demonstrated that marked or moderate activity of oestrus becomes, as the result of ovariectomy, progressively less and less, and is quite irregular within the space of two to four days. There is likewise a loss of heat concurrently with these changes. These findings are in agreement with the nineteen isolated observations on our other castrates, to which reference was made above.

3. *Substitution therapy with oestrus-producing substances.* The oestrus-producing substances which we have used are the water-soluble Theelin, and the lipoid-soluble Estrogen. These substances were supplied in ampoules, the former stated to contain 50 rat units, the latter, 25 rat units per cubic centimeter of solution. Estrogen is obtained in the lipoid phase resulting from suitable extraction of human placentae. Theelin is a crystalline substance, dissolved in water. It is extracted from human urine of pregnancy by the method of Veler, Thayer and Doisy (1930), and is prepared and supplied to us by the Department of Experimental Medicine, of Parke, Davis and Co. Both of these preparations have all of the oestrogenic properties ascribed to follicular fluid by many investigators in recent years. Allen and Doisy (1927) have reviewed the properties of such substances, as have Parkes (1929), and Frank (1929) more recently.

a. *Water-soluble oestrin: Theelin.* The rabbits which we have used in the Theelin series of experiments have been mentioned above. They were castrated one to fourteen days *post partum.* Normal, or control records were obtained about the fourth day subsequently, the Theelin injections were commenced immediately thereafter, when little or no

spontaneous activity was present. The dosage was administered in the desired number of rat units per kilogram body-weight. To obviate variable factors in the rate of absorption and inflammation at the site of injection, the intravenous route was used, and to retard wasteful elimination as much as possible, we have made four injections at two and one-half hour intervals, over a period of about eight hours in all. This has been an arbitrary and routine procedure to standardize the conditions as much as possible. In order that it might not be argued that we were using excessive quantities of Theelin, we have worked for minimal effects rather than maximal. We regard as important the fact that in no instance, in the recent castrates, has the sum-total of the four injections in any one doe exceeded one cubic centimeter of solution, or a fourth of a cubic centimeter per injection, and positive results have been obtained with as little as four injections of 0.006 cc. each. The single mass injection, at the termination of each experiment, however, did exceed one cubic centimeter of solution. When the smallest quantities of Theelin were used, it was measured accurately with a tuberculin syringe, and diluted with water. In no instance did the four injections of the dilution exceed 0.5 to 1.0 cc.

The general character of the response in these animals was most consistent throughout. In some instances there was the beginning of an increase in activity within six to ten hours which was at best only slight. By fifteen to twenty hours, considerably greater increase occurred. In all cases the maximum of activity was seen about twenty-four hours, or a little later, after the first injection of Theelin. Maximal activity continued in some instances until the second day, in others the peak of activity was over, and a subsidence of activity commenced. This decrease in activity proceeded at different rates in the several animals during the course of three to six or more days. When the activity had subsided until it resembled that seen before the injections, a second injection was made in most instances, to demonstrate that the uterus was still capable of a response. The second injection was a mass dose of usually 100 r. u. intravenously, and 100–200 r. u. subcutaneously. Records were obtained only once after such an injection, usually a day later, and just before the rabbit was killed and the uterus grossly examined for inflammation or infection. In our autopsy findings we have found local mucosal involvement in three instances. There is no reason to suspect, in these three cases, that the activity was modified by these pathological areas, inasmuch as infection in non-gravid uteri is, in our experience, accompanied by exaggeration of motility rather than a diminution. In none of these instances were the results anomalous, or different from those obtained in other rabbits whose uteri were found to be entirely normal in gross appearance.

Attention should be called to three exceptions (not those showing mucosal involvement) among our recent castrates which are possibly ex-

plicable on the basis of some variation in the value of the threshold dose. In two animals a total dose of 0.5 r. u. per kilo failed to give a response, even though one other animal in apparently a similar condition did give a response to this amount. It seems likely, therefore, that 0.5 r. u. per kilogram body-weight is sub-liminal for a definite response, although this cannot be definitely stated on so small a number of experiments. Two rat units per kilo regularly gave a response, however, and this amount is certainly above the threshold for a uterine response to Theelin. In yet another animal, the third exception, injected on the fourth day *post partum*, a dose of 5 r. u. per kilo gave no response, though two others at a similar period after parturition did respond to 15 r. u. per kilo. It is barely possible that some persistent effect from the recent pregnancy may still exert an influence on the first few days *post partum*, giving rise to a differential threshold to Theelin yet which is inoperative on the sixth day or

TABLE 3

Summary of data showing increase in uterine motility resulting from substitution therapy (Theelin): change in 24 hours

RECENT CASTRATES						OLD CASTRATES				
Increase in activity (irregular)	15 r.u. per kilo	5 r.u. per kilo	2 r.u. per kilo	0.5 r.u. per kilo	Total rabbits	Increase in activity	30 r.u. per kilo	20 r.u. per kilo	15 r.u. per kilo	Total rabbits
No change	0	1	0	2	3	No change	0	0	3	3
0 → +	0	1	0	0	1	0 → +	2	1	0	3
0, + → ++	0	2	2	1	5	0, + → ++	2	1	0	3
0, + → +++	2	2	3	0	7	0, + → +++	0	1	0	1
Total.....	2	6	5	3	16		4	3	3	10

later, when other recently castrated does responded with as little as 2 r. u. per kilo.

In general, our results on the old castrates are like those described for the recent castrates. The most apparent difference is not in latency,—which remains surprisingly constant,—but in the minimal effective dose required to elicit a response. We have obtained consistent positive responses in recent castrates with 2 r. u. per kilo, and in one instance with 0.5 r. u. per kilo. If we assume 2 rat units per kilogram body weight to be about the minimal effective dose, that for old castrates is found to be approximately ten times this amount, or between 15 and 20 r. u. per kilo. Three does gave negative results with 15 r. u. per kilo, whereas three others gave definite responses with 20 r. u. The only noticeable effect obtained with the smaller amount, 15 r. u., is that in one instance an irregular type of activity became regular within twenty-four hours, and a

good response was obtained after the mass injection which was made on the second day. A summary of the experiments indicating changes in motility as a result of Theelin administration is given in table 3.

b. *Lipoid-soluble oestrin: Estrogen.* Our experiments with Estrogen were not conducted with as rigorous a routine as were the Theelin experiments. Rabbits were used indiscriminately from our stock, irrespective of recent pregnancies. Single intravenous injections were made, and the number of days between castration and the day of injection was in most cases a matter of chance. Nevertheless, in spite of these irregularities in the routine, chief among these being the utter disregard of oestrus and anoestrus at the time of castration, the results are qualitatively alike among themselves, and are strikingly comparable in every way to the results which we have obtained with Theelin. In table 4 are listed three castrates which gave no response, whereas fourteen others did, with a small dosage in rat units per kilogram body-weight. These results,

TABLE 4

Summary of data showing increase in uterine motility resulting from substitution therapy (Estrogen), irrespective of dosage

CHANGE IN ACTIVITY	CASTRATED 2 TO 5 DAYS	CASTRATED 9 TO 16 DAYS	TOTAL RABBITS
No change	2	1	3
$0 \rightarrow +$	0	0	0
$0, + \rightarrow ++$	3	6	9
$0, + \rightarrow +++$	2	3	5
Total.....	7	10	17

obtained with what is considered a relatively crude extract which was not given under uniform conditions, is added evidence that it is the oestrus-producing substance or substances of placentae or urine of pregnancy that are effective in producing uterine activity in our castrate rabbits.

Discussion. *Oestrus and anoestrus.* If functional changes in the ovary exert a regulating influence on the motility of the uterus, along with controlling some of the known phenomena which are characteristic of sex behaviour in the female rabbit, then there are four conditions with which one should be able to correlate uterine motility. First, it is highly probable that during pregnancy the uterus is quiescent until the time of parturition, else abortion might occur spontaneously. The work of Knaus (1927) would support such a view, notwithstanding evidence to the contrary reported by Clark, Knaus and Parkes (1925–6). During a second condition in the rabbit, pseudopregnancy, the uterus is known to be quiescent until the termination of this period when uterine motility returns (Reynolds and Friedman, 1930a), and the doe comes again into heat.

A third condition of ovarian activity which might affect uterine motility is that of oestrus, and lastly, the condition of anoestrus might be expected to affect uterine motility in an opposite manner.

The periods at which female rabbits will copulate are varied and uncertain. Moreover, they will frequently accept the male under the most dissimilar conditions, and it is for this reason that we must arrive at a definition of the oestral condition, although it may be subject to change in the light of new work. Ordinarily, when a mature female rabbit copulates, a series of changes takes place within the animal which lead to ovulation in the course of ten or more hours. Sometimes, however, a non-pregnant female fails to ovulate in response to the stimulus of coitus; especially is this so in young does copulating for the first time. Pregnant or pseudopregnant does will accept the male sometimes, although ovulation, in the rabbit, is not known to occur at this time. The question arises, therefore, just which of these conditions may be considered oestral. Is the mere willingness of a doe to copulate indicative of oestrus, or is the willingness to copulate, plus the ability to ovulate, a more satisfactory definition of oestrus? The latter view is the one that appeals to us, and is the sense in which we have used the term "oestrus" in this work. Conversely, we regard anoestrus as a condition of unwillingness to mate in sexually mature non-pregnant, non-pseudopregnant rabbits which have functional ovaries. We realize that these definitions may not account for all the conditions one may see in the rabbit. For example, it is difficult to accept the condition we observed in a castrate doe as oestral, although this animal, on the second day of castration showed a moderate, regular activity of her uterine fistula, and exhibited all the accepted external signs of oestrus. One cannot say that she was not in heat, for she accepted the male four times in a few minutes, yet for our purposes it is impossible to consider that the condition of desire which she exhibited was necessarily comparable to oestrus in a normal, intact animal.

With the limitations of the definition stated above, it may be seen from our results, that in no case where marked uterine motility was seen was that doe not in oestrus. On the other hand, of twenty-three instances of does in anoestrus, seventeen of these showed only feeble and usually irregular activity, or else none at all. We are confronted with some irregularities, however, if indeed uterine motility *is* correlated with oestrus as we have defined it. One may see, in table 1, that of twenty one instances in which moderate activity was seen, approximately a third of these occurred during oestrus, the remaining two-thirds in anoestrus. Also, two does accepted the male while showing only feeble activity. Such irregularities are not easily explained. In our experience with mating rabbits we have frequently noted that of does which eventually accept the male, some copulate much more willingly than do others. Thus, in some

few cases, does may have coitus just once after many attempts on the part of the male. All subsequent attempts by the same buck or others are refused. More often, however, does will repeatedly accept the male, each time exhibiting marked lowering of the back, accompanied by elevation of the hind quarters and the tail reflex described by Parkes (1930a). It is indeed unfortunate that at the time when these apparent anomalies in our results presented themselves, no cognizance was taken of the eagerness, or lack of it, with which these does accepted the male. It is our impression, however, that when marked uterine activity is seen, the doe always shows great willingness to copulate. This is especially true of *post partum* does. It is barely possible, then, that some accounting for the irregular results may be made on this basis. Moreover, it must be emphasized that although our classification of activity is entirely arbitrary, any further subdivision does not clarify the situation at hand but, rather, complicates it. Nevertheless, despite the irregularities we have noted, we feel, in the light of the records we have seen, that the ovary is a factor of prime importance in regulating the motility of the non-gravid uterus of the rabbit, particularly in view of the striking correlation which exists between marked uterine activity and oestrus, and between little or no activity and anoestrus. Parkes points out (1929) that a general conclusion which may be drawn from other work on the excised uterus, is that feeble, rapid contractions are characteristic of dioestrus, while fewer but more powerful ones occur in oestrus. The work of Blair (1923), Keye (1923), Frank, Bonham and Gustavson (1925), and of Wislocki and Guttmacher (1924) support such a view. Clark, Knaus and Parkes (1925) however, find this generally to be the case, but state that no "certain" relationship between the stage of oestrus and the frequency of contractions exists, when records were made on the uterus of the anesthetized mouse and on the same uterus excised. Because of the irregularities in oestral activity which we have seen, however, we are obliged to add that there may be times, though rarely, when extraneous influences, which are at present unknown, modify or annul the effect of the ovary in the influence which it exerts on uterine motility. They may be nutritional, pathological, or operative, and in any event probably reside outside the ovary, since the rabbits showed other accepted signs of oestrus.

Castration. A still further line of evidence which supports the thesis that the ovary is of prime importance in regulating uterine motility, is to be found in the thirty-seven castration experiments reported above. We have seen that the complete absence of the immediate influence of the internal secretions of the ovary is associated with a partial loss at first, and a complete loss eventually, of all uterine motility. This occurs without exception, and as surely as atrophy of the uterus follows complete ovariectomy (Hammond and Marshall, 1925), although the loss of motility

appears to take place far more rapidly than does atrophy, and not *pari passu* with it. The experiments which we have performed on eight rabbits in which records of activity were obtained before castration, and on the days after it, leave no doubt that the effect may be attributed solely to the loss of ovarian influence.

Our results on the uterus *in vivo* lend support to the current idea, gained by studies with the uterus *in vitro*, that the uterus of the ovariectomized animal is quiescent. These effects have long been studied on the excised uterine strip. Athias (1919–20) reviews in detail the older literature bearing on this point, so that it need not be cited here. It will suffice to say that by the data obtainable by means of such technic, small undulating contractions are generally believed to characterize uterine movements in castrate animals. The results obtained by our method, and especially in those cases where records were obtained before and after castration, are in general agreement with the data obtained with other technics.

Replacement therapy. We regard the uniformity of the uterine response of the castrate to the injection of Theelin and Estrogen as being so regular and reproducible, that there can be little doubt but that the effects which we have observed are due to a potent substance in the oestrogenic extracts which we have used, and not to some non-specific effect. Moreover, it is our experience that saline, oxytocic substances or other extracts never induce activity in quiescent uteri of unanesthetized rabbits. Pituitrin, pitocin, gynergen (ergotamine tartrate) and histamine (0.025–0.01 mgm.) are impotent on all quiescent uteri of unanesthetized rabbits where we have tried them. Furthermore, in work soon to be reported (Weinstein, Reynolds and Friedman, 1931), we have been unable to produce an anaphylactic response in sensitized quiescent uteri, although a response can readily be obtained in the sensitized active uterus. It is, therefore, no doubt significant that, in our experience, Theelin and Estrogen are the *only* agents by which activity in inactive uteri has ever been induced.

We believe that the long latency which we have invariably obtained presents one of the more important aspects of these results with oestrin on the uterus *in vivo*. There is, however, some recent work which purports to show that oestrin has an *immediate* effect upon the uterus *in vitro*. We do not deny that this is so, but we do doubt the physiological significance of it. In our work we have seen that a full half-day elapses before there is any appreciable effect to be noted in the response to liminal amounts of Theelin, and it is twenty-four hours or more before the peak of activity is over. A latency of this duration is not unknown in other humoral mechanisms in the rabbit. Ovulation, which depends upon a humoral mechanism (Friedman, 1929), requires over ten hours. Our results, therefore, are in striking contrast to those of Brouha and Simonnet (1927a; 1927b) who report that a contracting uterus from an animal in heat becomes quiescent

by merely washing it for twenty minutes with an oestrin-free perfusion fluid, and that the addition of oestrin to any quiescent uterus promptly initiates activity. Bourne and Burn (1928) however, find that only "especially sensitive" uteri *in vitro* will respond to oestrin, and Fraenkel (1927) reports completely negative results. Durrant (1930), moreover, finds that commercial oestrogenic extracts diminish or even abolish activity of the uterus *in vitro*. Because of these contradictions, and because of the immediate effect of oestrin on the excised uterus, one wonders as to their significance, especially in the light of the excellent work of Van Dyke and Hastings (1928) who have shown the profound influence of ionic imbalance on the motility of the excised uterus, particularly of the calcium ion. From the work of the latter investigators, it appears that results obtained in response to the addition of drugs and extracts to the fluid which bathes an excised uterine strip cannot have real physiological significance unless the ionic environment of the tissue be quantitatively controlled, both before and during the experiment. Our work, therefore, would appear to substantiate the work of some of these other investigators, but we believe that because we have demonstrated, on one and the same uterus *in vivo*, the response of the oestrogenic extract we have used, that our results present a physiological picture that may not be justifiably inferred from theirs, and hence the agreement is more apparent than real.

The finding that oestrin will produce activity in the quiescent uterus appears to present a paradox, namely, that this substance which produces and sometimes regulates uterine contractions is obtained from human urine of pregnancy, yet it does not produce abortion in the woman from whom it was obtained. We must suppose therefore, that its effect on the human uterus is of so little consequence that it will not terminate pregnancy, or else that it is directly or indirectly rendered ineffective. There is some evidence that the latter view may be correct. One of the properties of Theelin is that its effect even in minute quantities, is prolonged over many hours, or even days. If then, we may assume that Theelin is identical with the oestrus hormone or hormones of the rabbit, we must admit that the rapid cessation of uterine motility that occurs within a few hours after coitus (Reynolds and Friedman, 1930a) is due either to an alteration in the effectiveness of the oestrus hormone, or to a lowered renal threshold that permits its rapid elimination from the blood stream. It has been amply demonstrated however, that during the latter half of pregnancy in the human, there are far larger quantities of oestrin in both the blood and urine than at other times (Ascheim and Zondek, 1927; Laquer, 1927; Slotta, 1927; Veler and Doisy, 1928). This can only mean that the human uterus is quiescent in spite of a great concentration of oestrin in the blood, if the human uterus behaves as does the uterus of the rabbit in this respect. From these considerations, therefore, it seems likely that oestrin is rendered

ineffective during pregnancy, as regards uterine motility. This is further borne out by the observation that 5 cc. of whole human urine of pregnancy inhibit uterine motility within five to eight hours after it is injected intravenously into a doe showing marked uterine activity (Reynolds and Friedman, 1930b). This happens in spite of the fact that there is a potent oestrus producing substance in the urine plus that presumably present also in the blood of the rabbit. We have roughly estimated that the 5 cc. of urine which we injected contained oestrin in amounts about equivalent to the minimal effective quantity noted above, in the recent castrate experiments.

The literature does not afford much help on this seeming paradox, inasmuch as heretofore there has been no way to estimate the nature of the action of oestrin in non-pregnant animals. Parkes (1930b) very recently has reviewed the literature, and extended the observations on the effect of the water soluble oestrin in the course of pregnancy in experimental animals. He found that it was possible to produce abortion in eighteen out of twenty-four mice by the injection of a total of 360 m. u. (approximately 48 r. u.) of oestrin in the course of thirty-six hours. Smaller quantities, 260 m. u. (32 r. u.) would not regularly produce abortion. Moreover, Fraenkel (1927) was unable to interrupt pregnancy in the rabbit, and Loeb and Kountz (1928) report negative results in the guinea pig. The difference may be one of species difference, or what is more likely, only excessive or toxic amounts of oestrin are effective. Considered in this light, these data may support the suggestion that oestrin in the pregnant animal is rendered ineffective in its effect on the motility of the uterus. Only quantitative studies on the uterus *in vivo* will supply an answer to this question. Such experiments on the unanesthetized, pseudopregnant rabbit are being carried out in this laboratory at the present time.

It is a pleasure to acknowledge my indebtedness to Dr. Maurice H. Friedman, who first suggested the uterine fistula preparation and its application to this and other problems, for the many kindly criticisms and suggestions he has given me.

CONCLUSIONS

1. Marked uterine activity in the non-pregnant rabbit is associated with oestrus. Feeble activity has been twice seen in oestrus; moderate activity has been seen seven times. Anoestrus is usually associated with only feeble activity, or none at all.

2. The motility of the uterus of a rabbit deprived of its ovaries during oestrus becomes irregular and progressively less in two to four days. Complete quiescence eventually supervenes. The change has been

demonstrated in the same rabbit, by obtaining records both before and after castration.

3. Replacement therapy in castrate rabbits with Theelin and Estrogen, commercial water and lipoid soluble oestrogenic extracts, may impart to the quiescent uterus of those animals, activity such as usually occurs in the presence of the ovaries during oestrus.

4. Because of the conclusions noted in 1, 2 and 3 above, we feel justified in concluding that the ovary of the non-pregnant rabbit normally exerts a profound effect upon the motility of the uterus.

5. Due to the occasional irregularities in uterine motility seen during oestrus, mentioned above, it appears that certain unknown extra-ovarian influences sometimes, though rarely, modify or completely annul the effect of the ovary on the motility of the uterus.

6. The minimal effective dose of Theelin administered as we have used it, is less than 2 rat units per kilogram bodyweight in recently castrated rabbits. Twenty rat units per kilo is the minimal effective dose in rabbits ovariectomized for three weeks.

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